

<b>TITLE: Microbiology Specimen Collection</b>	
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**PURPOSE:**

The purpose of this procedure is to provide instructions for the collection of Microbiology specimens to the nursing staff.

**RESPONSIBLE PARTIES**

- Nursing staff

**ATTACHMENTS:**

- Microbiology Specimen Collection Guide

**PROCEDURE:**

See table of contents for desired procedure and CTRL + click to follow link.

## Microbiology Collection Guide

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## **GENERAL INFORMATION**

1. Obtain specimens for culture prior to antibiotic administration if possible.
2. If patient is already receiving anti-microbial therapy, list agents on the request slip.

## **HANDLING PRECAUTIONS:**

1. Observe Universal Precautions when collecting or handling specimens. See "Hospital-Wide Policy" for prevention of Blood Borne Diseases (Lab Safety Manual).
2. If a specimen is accidentally spilled, cover the contaminated area with paper towels. Wipe up gross contamination immediately with a gloved hand and dispose in a biohazard bag.
3. Call Environmental Services to decontaminate the affected area. If Environmental Services is not available, decontaminate area by saturating a paper towel with germicidal and clean the area.
4. If a specimen container has been externally contaminated by blood or body fluid, transfer to clean container if possible. If transfer to another container is not possible, clean container with germicidal and place in a clear plastic bag. Label CONTAMINATED before transporting to lab.

## **COLLECTION AND TRANSPORT:**

1. Use appropriate collection and transport containers for specimen collection. See individual procedures for preferred transport containers.
2. Seal containers before transporting to lab. Transport containers are available from Materials Management or Microbiology. Note expiration date.
3. Carefully follow directions on BBL Vacutainer Brand Anaerobic Specimen Collector. Do not remove the inner tube from the outer unit on BBL anaerobic transport tubes.
4. Transport sputum collection containers intact. Tape lid onto outer unit.  
**NOTE: PLACE ALL SPECIMENS IN A ZIP-LOCK BAG BEFORE TRANSPORTING TO LABORATORY.**
5. Transport Viral culture specimens in Universal Transport Media (UTM). UTM is available in the Laboratory. Store at room temperature. Media is stable until expiration date written on the tube.

## **LABELING:**

1. Specimens received for culture from hospital or outpatients must contain the following Information:
  - A. Patient's name, first and last
  - B. Date and time of collection
  - C. Medical Records number (inpatients) and date of birth (outpatients)
  - D. Source & test wanted

### **REQUEST FORMS:**

1. Inpatients: ORDER TESTS IN CERNER
2. Outpatient clinics:  
Complete forms with NAME, DOB, SEX, #, DATE AND TIME of collection, INITIALS of person collecting specimen, DX., ANTIBIOTICS, PHYSICIAN and SOURCE.
3. Viral cultures must be accompanied by a Northern Regional Lab (State Lab) Request Form that is completely filled out. State Lab Request Forms are available in Microbiology. State Lab will not process specimens unless all forms are complete.

### **DELIVERY TO LAB:**

1. Deliver specimens to lab immediately after collection.
2. See directions for specimen storage under each procedure.

### **CRITERIA FOR REJECTION OF SPECIMENS:**

1. Specimens accompanied by slips without DATE and TIME of collection will not be processed until that information is acquired.
2. Sputum:
  - A. Routine and Fungal cultures:
    1. Contaminated outer containers **ARE NOT** acceptable. Specimens with many epithelial cells indicate gross contamination with saliva. Organisms isolated from these specimens are not indicative of infection.
    2. Microbiology department evaluates specimen for acceptability: all specimens having more than 40 epithelial cells will not be cultured. Specimen will be rejected for culture and a repeat specimen will be requested by phone.
    3. Delay in transport (greater than 2 hours at room temperature) is not acceptable.
  - B. AFB Cultures:
    1. Contaminated outer containers are not acceptable.
    2. More than one specimen submitted per day. State Lab will accept samples collected on the same day if they meet the following criteria, at least one sample is collected as a first morning sample, all samples are collected at least 8 hours apart, with the dates and times clearly marked on the container and on the requisition.
      - a. Sputa for AFB cultures must be refrigerated if there is a delay in transport.
      - b. Recovery of AFB is maximized by collecting an early A.M. specimen.
      - c. 5ml of specimen is required.
3. Urine:
  1. Delay in transport (greater than 1/2 hour unrefrigerated). Refrigerated urine specimens are acceptable up to 24 hours after collection. However, prompt delivery facilitates timely results. Specimens collected in Becton Dickinson (gray top tube) are acceptable for culture up to 48 hours after collection, without refrigeration.
  2. FOLEY CATH TIPS ARE NOT ACCEPTABLE FOR CULTURE.
  3. Specimens contaminated by fecal contents.

4. Stool Specimens:
  - A. Routine Cultures:
    - a. Contaminated outer container
    - b. Specimens in diapers
    - c. Specimen not in transport media for more than 2 hours  
Transport media is used to deter the overgrowth of non-pathogens and to insure the survival of enteric pathogens.
  - B. Clostridium difficile:
    - a. Contaminated outer container
    - b. Delay in delivery to Lab (longer than 1 hour)
    - c. Specimen submitted in transport media, specimen should be in sterile container only.
  - C. Ova and Parasites (O&P)
    - a. Contaminated outer container
    - b. Specimen not in transport media. (Transport media is available from Materials Management and the Microbiology Lab.)
    - c. Stool from patients recently administered barium enemas, mineral oil, bismuth or magnesia cathartics.
    - d. More than one specimen submitted per day.
5. Tissue Specimens—specimens submitted in formalin are unacceptable.
6. Other Specimens:
  1. Improperly handled anaerobic transport tubes. Indicator shows anaerobic conditions have not been maintained.
  2. Specimens received in a syringe with attached needle **ARE NOT** acceptable. Please transfer specimens to appropriate containers prior to transport.
7. New specimens will be requested. Original specimens will be held until a replacement arrives. Except in cases of gross contamination, requests for processing of unacceptable specimens by physicians will be honored. However, specimen condition will be noted under "Comments" on the report.

# MICROBIOLOGY REQUEST POLICIES

## BIOPSY AND TISSUE

### **MATERIALS:**

1. Sterile container without preservative.

### **PROCEDURE NOTES:**

Tissue specimens are cultured both AEROBICALLY and ANAEROBICALLY.

### **PROCEDURE:**

2. Place specimen in sterile jar or in a BD Anaerobic transport tube.
3. If tissue is transported rapidly, it will protect anaerobes.

### **TRANSPORT AND STORAGE:**

1. Take specimen to lab IMMEDIATELY.
2. Keep at room temperature.
3. Specimens must be accompanied by a completed Microbiology Request Form.

## BLOOD CULTURE:

### **Guidelines for Blood Culture Collection:**

1. Studies at Mayo Clinic and elsewhere have shown that the detection of bacterial sepsis increases with the amount of blood drawn and that THREE blood cultures are optimal for detection of septicemia. Therefore, three sets of blood cultures per day per patient are recommended. Some acceptable reasons for drawing more than three per day include:
  - a. A new episode of suspected sepsis generated by a condition not present when first cultures were drawn.
  - b. Suspected subacute bacterial endocarditis
  - c. An unusual clinical situation.
2. If the drawing intervals are specified by the physician, they will be respected. However, the following drawing protocols are recommended:
  - a. For specimen being taken to rule out bacteremia with the mildly febrile patient: Draw specimens at intervals of *one hour apart or longer*.
  - b. For the critically septic patient for whom the over-riding concern is to start antibiotic therapy quickly: Draw two blood cultures, one right after the other.
3. Blood culture set includes a FAN aerobic bottle plus an ANA anaerobic bottle. The FAN bottle contains 22 ml of complex media and 8 ml of a charcoal suspension, which removes antibiotics that may inhibit the growth of organisms.

### **MATERIALS:**

1. ChloroPrep One-Step sponge.
2. Sterile 20cc Syringe
3. 2 Sterile needles
4. 2 Blood Culture Bottles
5. Tourniquet

### **PROCEDURE NOTES:**

1. Due to the increased risk of contamination of blood cultures, the lab encourages Nursing Services to request blood cultures to be drawn by Lab personnel.
2. When drawing INFANTS or CHILDREN under 10, draw approximately 1 ml for each year of life.
3. If less than 5 ml is drawn, place the entire amount into FAN bottle.

### **PROCEDURE:**

- A. Remove blood culture bottle caps. Place alcohol pad on top of the bottle.
- B. Apply tourniquet to patient's arm showing the most promising veins. Palpate area to locate vein.
- C. Release tourniquet.
- D. Sterilize venipuncture site by cleaning with Chloraprep One-Step applicator sponge in concentric circles. Allow to dry at least 2 minutes. Reapply tourniquet.
- E. Do not touch venipuncture site at this point unless sterile gloves are worn.
- F. Assemble the needle, syringe, tourniquet, and gauze pad near the patient's arm. Put on vinyl gloves.
- G. Immobilize the vein and perform venipuncture, drawing 16 to 20cc of blood.
- H. Recommended total volume of blood cultures:
  1. Neonates to 1 year (<4 kg): 0.5 to 1.5 ml per tube (at least 1 ml is preferred)
  2. 1 to 6 years: 1 ml per year of age
  3. Children weighing 30 to 80 lbs: 5-10 ml per set
  4. Adults and children weighing >80 lbs: 15-20 ml per set
- I. Inject 8-10 ml of blood into each bottle without allowing air to enter the bottles. Label the bottles with patient name, date, time, site of collection, and the initials of the phlebotomist performing the procedure.
- J. When drawing children under the age of 10, draw approximately 1 ml of blood for each year of life. If less than 5 ml is drawn, place entire amount into the FAN bottle.
- K. Should the vein be missed, use a new needle and re-sterilize the site.
- L. After completion of the venipuncture apply a bandage to the site after bleeding has stopped

### **TRANSPORT AND STORAGE:**

1. Deliver blood culture bottles to lab IMMEDIATELY.
2. Every effort should be made to deliver bottles so that appropriate incubation conditions can be met ASAP. If there is any delay, STORE at room temperature.
3. Microbiology slip must be properly filled out and include the collection time.
4. If anaerobes are suspected, it must be noted on the requisition.

## **BODY FLUIDS**

### **(PERITONEAL, ASCITES, PARACENTESIS, JOINT FLUID)**

#### **MATERIALS:**

1. Acu-dyne/alcohol
2. Sterile syringe and needle
3. MB Request slip
4. BD Anaerobic Transport Tube\* Do not transport specimen in syringe and needle.

#### **PROCEDURE:**

1. Cleanse skin with antiseptic and collect via percutaneous aspiration. Body fluids often contain anaerobes which are not viable after exposure to oxygen.
2. Place fluid in BD anaerobic transport tube (discard swab), following directions on the package. Label specimen with Patient's NAME, DATE, and SOURCE of specimen.

#### **TRANSPORT AND STORAGE:**

1. Transport specimen to lab IMMEDIATELY.
2. Keep at room temperature.
3. Specimen must be accompanied by a completed Microbiology request slip.

## **CONTINUOUS AMBULATORY PERITONEAL DIALYSIS FLUID**

1. Enclose dialysate bag in a larger plastic bag. Place this bag into a disposable plastic pan, and transport it to the laboratory.
2. Specimen must be accompanied by a completed Microbiology request slip.

## **CEREBROSPINAL FLUID (CSF)**

### **MATERIALS**

1. 3-4 CSF collection tubes
2. Spinal needle and other materials needed for local anesthesia and for disinfecting the puncture site
3. Sterile gloves
4. Antiseptic - Betadine

### **NOTES:**

1. Several organisms, e.g., *N. meningitidis* and *H. influenzae*, can be lost at refrigerator temperatures.

### **COLLECTION:**

1. CSF is collected by the physician. Care must be taken to disinfect the skin in the area to be sampled.
2. Generally 10 ml is taken and divided into 3-4 tubes.
3. Generally, specimens submitted for culture should be the 3rd tube collected since residual skin contaminants are more likely to be present in the initial volume.
4. Label Specimen.

### **TRANSPORT AND STORAGE:**

1. Transport specimen to lab IMMEDIATELY with appropriate request forms.
2. Testing is usually a STAT procedure.
3. Keep specimens at room temperature.
4. The microbiology tests that can be ordered on CSF are:

• Gram stain (can be STAT)	Meningitis Panel and Group B Strep test are SEND OUT
• Routine culture	
• Fungal culture	
• AFB culture	
• AFB stain	
• Crypto-latex test* for <i>Cryptococcus</i> (can be STAT) *India Ink test is not longer done for <i>Cryptococcus</i> . Crypto-Latex test is much more sensitive.	

## **DRAINAGE AND EXUDATES**

### **MATERIALS**

1. BD Anaerobic Transport
2. Sterile syringe and needle

### **PROCEDURE NOTES:**

Drainage and exudates should be cultured both AEROBICALLY and ANAEROBICALLY. All organisms survive in the BD ANAEROBIC Transport. Please take care to use proper techniques to preserve anaerobes.

### **PROCEDURE:**

1. If there is abundant exudate or drainage, discard the portion near the surface and collect material representative of the deepest portion of active margin of the site.
2. Aspirate using a needle and syringe. Place aspirate into BD anaerobic transport (discard swab) following directions on the package.
3. Specimens received in Aerobic CultureSwab are not acceptable for anaerobic cultures and will only be performed upon doctor's request. A comment will be added to these cultures that recovery of anaerobes will be compromised.
4. Swab specimens are NOT recommended, because they are difficult to obtain without contaminating the specimen.

### **TRANSPORT AND STORAGE:**

1. Take specimen to lab IMMEDIATELY.
2. Keep at room temperature.

## **EAR CULTURE**

### **MATERIALS:**

1. Culturette (for outer ear)
2. Needle and syringe (inner ear)
3. BD Anaerobic Transport

### **PROCEDURE:**

1. EAR CANAL, OUTER EAR:
  - A. Collect specimen with swab from a culturette.
  - B. Sample the active margin or deepest portion of the infected area. As much as possible, avoid sampling areas of healing and non-infected tissue.
  - C. Place swab in culturette. Crush ampule.
2. MIDDLE EAR:
  - A. Specimen is obtained by physician.
  - B. If perforation of the eardrum has not occurred, aspirate through the tympanic membrane using a needle and syringe.
  - C. Place contents of syringe in BD anaerobic transport vial.
  - D. If perforation has occurred, use a swab to remove and aseptically discard the discharge in the upper ear canal and cerumen.
  - E. With a second swab, collect the discharge nearest the eardrum.
  - F. Place swab in culturette if only aerobic organisms are suspected. A BD Anaerobic Transport Vial can be used for BOTH aerobic and anaerobic organisms. However, an aerobic CultureSwab is not acceptable transport for anaerobic organisms.

### **TRANSPORT AND STORAGE:**

1. Take labeled specimen to lab IMMEDIATELY.
2. Keep specimen at room temperature.

## **EYE CULTURES**

### **MATERIALS:**

1. Culturette
2. Sterile rayon tipped applicator (SRTA)
3. BD Anaerobic Transport (if anaerobes are suspected or requested.)
4. Needle and syringe (for aspirate specimens)

### **PROCEDURE:**

1. CONJUNCTIVAL SPECIMENS
  - A. Collect specimens with a nasopharyngeal swab (Rayon swab).
  - B. Sample from the deepest part of the infected area.
  - C. Place swab in culturette and crush the ampule.
  - D. If an anaerobic culture is desired, physician should collect specimen with a needle and syringe. Place the specimen in a BD anaerobic transport vial.
2. CORNEAL SCRAPINGS FOR KERATITIS
  - A. Collected by an ophthalmologist
  - B. Recommended anesthesia is 1-2 drops of proparacaine hydrochloride. This anesthetic is less inhibitory to bacteria than other compounds.
  - C. CONTACT LAB PRIOR TO PROCEDURE. Media for culture and slides for gram stain for direct inoculation is required.
  - D. Multiple scrapings with a platinum spatula are recommended. Each scraping should be placed directly on the media or on a slide for gram stain.
3. INNER EYE INFECTIONS
  - A. Procedure is done in the OR.
  - B. Requires procurement of intraocular fluid by an ophthalmologist
  - C. Needle and syringe aspiration is an acceptable method to collect specimens.
  - D. Place contents in a BD anaerobic Transport Vial.

### **TRANSPORT AND STORAGE:**

1. Specimens must be taken to the lab IMMEDIATELY.
2. Keep specimen at room temperature.
3. Be sure specimen is properly labeled and accompanied by a completed Microbiology Request Form.

## **FUNGAL CULTURES**

### **ROUTINE**

#### **MATERIALS:**

1. Sterile Petri dish (from Microbiology) or other sterile container.
2. Microscope slide
3. Scalpel

#### **PROCEDURE NOTES:**

1. KOH will be done upon request.
2. Specimen must be accompanied by a Microbiology Request form marked FUNGAL.

#### **COLLECTION, TRANSPORT & STORAGE:**

1. HAIR
  - A. Select hair that is broken and/or fluoresces under Wood's light.
  - B. Send at least 6-10 affected hair strands in Petri dish or other sterile container. Label specimen.
  - C. Take to lab as soon as possible. Keep at room temperature.
2. SKIN SCRAPINGS
  - A. Hold an open Petri dish under the lesion and scrape entire periphery of lesion using a microscope slide or scalpel.
  - B. Secure lid on Petri dish with tape. Label specimen.
  - C. Take to lab as soon as possible. Keep at room temperature.
3. NAILS
  - A. Using a scalpel or microscope slide, place debris under infected nail and/or scrapings through the diseased portion in a Petri dish.
  - B. Secure lid on Petri dish with tape. Label specimen.
  - C. Take to Lab as soon as possible. Keep at room temperature.

### **FUNGAL CULTURES—RINGWORM LESIONS**

#### **FUNGAL CULTURES—SUSPECTED SPOROTRICHOTIC ULCER**

#### **MATERIALS:**

1. 2-3 Culturettes

#### **COLLECTION, TRANSPORT AND STORAGE:**

1. Collect 2-3 swabs of exudate
2. Place in culturette. Break ampules.
3. Label specimens. Take to lab as soon as possible. Keep at room temperature.

### **FUNGAL CULTURES—BIOPSY OF SKIN ULCER**

#### **MATERIALS:**

1. Sterile container for transport.

#### **COLLECTION, TRANSPORT AND STORAGE**

1. Biopsy lesion to include wall and base.

2. Place specimen in container. Label.
3. Take specimen to lab as soon as possible. Store at refrigerator temperature.

### **FUNGAL CULTURES—SPUTUM**

#### **MATERIALS:**

1. Sputum collection kit
2. Glass of water

#### **PROCEDURE NOTES:**

1. Sputum, Bronchial Brushes, and Bronchial Washes can be used to detect lung lesions caused by fungus or yeast.
2. A properly filled out Microbiology Request Form must accompany all specimens.

#### **PROCEDURE, TRANSPORT AND STORAGE:**

1. Have patient collect an early morning specimen, after washing mouth with water.
2. Leave collection container intact.
3. Seal with tape. Label specimen.
4. Take to lab immediately.
5. Keep at refrigerator temperature.

### **FUNGAL CULTURES-PNEUMOCYSTIS**

#### **PROCEDURE:**

1. Collect specimen as above.
2. Fill out Microbiology Requisition. There is a Specific Test Request on this form for Pneumocystis.
3. Send to Laboratory ASAP.
4. Specimen MUST be kept refrigerated.

### **FUNGAL CULTURES—BRONCHIAL BRUSHES, WASHINGS**

#### **PROCEDURE:**

1. Specimen is obtained by the physician.
2. Label specimen and take to lab IMMEDIATELY.
3. Keep bronch brushes at room temperature.
4. Keep Bronch washings at refrigerator temperature.

### **FUNGAL CULTURES—ABSCESSES**

#### **MATERIALS:**

1. Syringe and needle
2. Sterile tube

#### **PROCEDURE NOTE:**

If specimen is for both BACTERIAL and FUNGAL cultures, follow directions for BACTERIAL COLLECTION.

#### **PROCEDURE, TRANSPORT, AND STORAGE:**

1. Aspirate from non-draining lesion.
2. Obtain pus plus a portion of the wall of the abscess, if possible.

3. Place specimen in a sterile tube.
4. Label specimen. Take to lab IMMEDIATELY.
5. Keep at room temperature.

### **FUNGAL CULTURE—TISSUE**

#### **MATERIALS:**

1. Sterile container

#### **PROCEDURE, TRANSPORT AND STORAGE:**

1. Obtain both wall and center of lesion.
2. Place in sterile container.
3. Label specimen and take to lab IMMEDIATELY.
4. Keep at room temperature.

### **FUNGAL CULTURES—BLOOD**

#### **MATERIALS:**

See method for routine blood culture collection.

#### **PROCEDURE NOTES:**

1. All specimens must be accompanied by a properly completed Microbiology Request Form.
2. Blood cultures for fungus are held for 10 days before being discarded as negative.

#### **COLLECTION, TRANSPORT, AND STORAGE:**

1. Collect using the same techniques and media bottles as for routine blood bacterial blood culture.
2. Label specimen. Transport to lab as soon as possible.
3. Keep at room temperature.

### **FUNGAL CULTURE—URINE**

#### **MATERIALS:**

1. Sterile urine cup
2. Equipment for clean catch urine

#### **COLLECTION, TRANSPORT AND STORAGE:**

1. See routine bacterial urine collection procedure for instructions to patient.
2. Label specimen. Take to lab immediately.
3. Keep at refrigerator temperature.

### **FUNGAL CULTURES—CEREBROSPINAL FLUID**

#### **MATERIALS:**

Same as bacterial collection

#### **PROCEDURE NOTE:**

India Ink tests for cryptococcus have been replaced by the cryptococcal antigen test which is more sensitive. This test is done on all CSF fungal cultures.

#### **COLLECTION, TRANSPORT AND STORAGE:**

1. Obtain 1-3 ml CSF.
2. Label specimen and take to lab IMMEDIATELY.
3. Keep at room temperature.

## **GENITAL TRACT CULTURES**

### **GENITAL TRACT—ROUTINE CULTURES**

#### **MATERIALS:**

1. Culturette
2. Sterile Rayon Tipped Applicator

#### **PROCEDURE NOTES:**

1. Routine cultures include detection of most common pathogens such as Yeast, N. Gonorrhoeae, Staph aureus, Group B streptococci, Gardnerella vaginalis, and any other predominant organism not considered usual flora.
2. Label specimen with patient's NAME, DATE and TIME.
3. Since "ROUTINE" cultures include detection of gonococci, rapid transport to the lab is necessary to maintain viability.
4. Time of collection is needed for QA purposes.

#### **PROCEDURE:**

1. MALE:
  - A. Urethral specimens should not be collected until AT LEAST one hour AFTER urination.
  - B. Urethral discharge can be collected on a culturette swab. Place swab in culturette and break ampule.
  - C. If no discharge is obtained, a Sterile Rayon Tipped Swab, should be inserted into the distal urethra for approx. 2 cm. and gently rotated.
  - D. Place swab in culturette and break ampule.
2. FEMALE
  - A. Vaginal specimens for vaginitis
    1. Obtain specimen by collecting ample amounts of discharge from the vaginal wall.
    2. Place swab in culturette and crush ampule.
    3. Vaginal specimens are NOT acceptable for the detection of N. gonorrhoeae.
  - B. Endocervical specimens:
    1. Moisten speculum with warm water or saline. DO NOT use any other lubricant.
    2. Wipe cervix clean with cotton swabs to remove vaginal secretions.
    3. Collect discharge on a swab by using a ringing motion to help force exudate from the cervical glands.
    4. If no exudate is observed, insert swab into endocervical canal. Move from side to side and allow 30 seconds for absorption of organisms to swab.
    5. Place swab in culturette and crush ampule.

#### **TRANSPORT AND STORAGE:**

1. Take culturette to lab IMMEDIATELY.
2. Store at room temperature
3. Fill out Microbiology Request Form. Include TIME of collection.

## **GENITAL TRACT—'GC' ONLY CULTURES**

### **MATERIALS:**

1. Rayon Swab.
2. Thayer Martin/Chocolate Agar (TM/CA) pill pocket plate or a Thayer Martin plate plus a Chocolate plate that is at room temperature.
3. CO2 generating pill
4. Plastic zip-lock bag

### **PROCEDURE NOTES:**

1. Cotton swabs contain fatty acids that can be inhibitory to gonococci.
2. Pill pocket plates with zip-lock bag are available in the Microbiology Lab.
3. GC can die quickly. Proper use of CO2 pill pocket plates and immediate transport to lab facilitate optimum recovery.
4. IMMEDIATE transport to the lab is important so that the conditions for viability of the gonococci can be met.
5. Time of collection is needed for QA purposes.

### **PROCEDURE:**

1. MALE:
  - A. Urethral specimens should not be collected until AT LEAST one hour AFTER urination.
  - B. Urethral discharge can be collected on a swab. Roll swab firmly onto both sides of the TM/CA media or Thayer Martin plate plus a Chocolate plate.
  - C. If no discharge is obtained, a Sterile Rayon Tipped Swab should be inserted into the distal urethra for approximately 2 cm. and gently rotated. Withdraw and roll swab onto both sides of the TM/CA media or Thayer Martin plate plus a Chocolate plate.
2. FEMALE:
  1. Moisten speculum with warm water or saline. Do not use any other lubricant.
  2. Wipe cervix clean with cotton swabs to remove vaginal secretions.
  3. Collect discharge on swab by using a ringing motion to help force exudate from the endocervical glands.
  4. If no exudate is observed, insert swab into endocervical canal. Move from side to side and allow 30 sec. for absorption of organisms onto swab.
  5. Roll swab onto both sides of the ML/CA media or Thayer Martin plate plus a Chocolate plate..
3. PROCEDURE FOR ANORECTAL SPECIMENS
  - A. Insert swabs 4-5 cm into anal canal. Move swab side to side to sample crypts.
  - B. If fecal contamination occurs, discard swabs and use another to obtain specimen.
  - C. Roll swab onto both sides of the ML/CA media or Thayer Martin plate plus a Chocolate plate..

### **TRANSPORT AND STORAGE:**

1. IMMEDIATELY place CO2 pill into "pocket". Label plate and place in ziplock bag. Seal tightly. Take specimen to lab IMMEDIATELY.
2. Keep at room temperature.
3. Fill out Microbiology Request Form and include TIME of collection.

**GRAM STAIN:**

Gram stains can be a rapid means of diagnosing presumptive GC in MALES. However, they are NOT recommended in FEMALES because 50% of the culture positives can be missed. If a gram stain is done on a FEMALE for GC, it always should be followed by a culture.

**GENITAL TRACT—GROUP B STREP SCREEN—CULTURE****MATERIALS:**

1. Culturette

**PROCEDURE NOTES:**

1. Culturettes will maintain Group B Strep for several hours. However, prompt delivery allows for earlier recovery of organisms.
2. Specimen must be accompanied by a properly completed Microbiology Request Form.

**PROCEDURE:**

1. Collect a mucosal swab.
2. Place swab in culturette. Crush ampule.

**TRANSPORT AND STORAGE:**

1. Transport to lab IMMEDIATELY.
2. Keep at room temperature.
3. Label with patient's NAME and DATE and source.

**Vaginal/Rectal Swabs:**

1. One or two swabs may be used.
2. Collect a vaginal swab as above.
3. Use a second swab, or the same swab, and pass the tip app. 1 in. beyond the anal sphincter. Carefully rotate the swab to sample the anal crypts, and withdraw the swab.
4. Send the swab, in the culturette, with the end crushed, to the lab as soon as possible.

**BARTHOLIN GLAND ABSCESS AND OTHER GENITAL CULTURES THAT INCLUDE PUS, FLUID AND TISSUE****MATERIALS:**

Culturette (if no anaerobe is suspected)

BD Anaerobic Transport Vial Anaerobic transport can be used for both aerobic and anaerobic cultures

**PROCEDURE NOTES:**

Anaerobic Transport will keep anaerobes viable for several hours.

Specimen must be accompanied by a properly completed Microbiology Requisition.

**PROCEDURE:**

Collect draining pus from Bartholin duct with sterile swabs.

If no pus can be expressed, aspirate with needle and syringe. Place specimen in BD anaerobic transport vial following the directions on the package.

**TRANSPORT AND STORAGE:**

Transport specimen to lab IMMEDIATELY.  
Keep at room temperature.

**GENITAL CULTURES—IUD AND OTHER CULTURES FOR ACTINOMYCES**

**MATERIALS:**

1. Thioglycolate Broth for IUD (MB)
2. BD Anaerobic Transport for other specimens (MB). A sterile Petri dish or sterile container can be used for transporting an IUD. However, Thioglycolate Broth is preferred.

**PROCEDURE:**

1. Place IUD in Thioglycolate broth or in sterile Petri dish.
2. Place other specimens in Anaerobic transport following the directions on the package.
3. Actinomyces cultures are held for 14 days before discarding as negative.

**TRANSPORT AND STORAGE:**

1. If IUD is NOT transported in Thio, it must be taken to lab IMMEDIATELY.
2. Transport Thio Broth or Anaerobic Transport to Lab ASAP.
3. Store at room temperature.

**GENITAL CULTURES - HERPES CULTURES**

**MATERIALS:**

1. 26 or 27 gauge needle and tuberculin syringe for vesicular fluid aspiration
2. Non-Calcium alginate swab
3. Sterile scalpel for lesion scrapings
4. Universal Transport Media (UTM) (in laboratory)

**PROCEDURE NOTES:**

1. Herpes cultures are referred to the Northern Regional Lab in Fairbanks.
2. UTM is good until the expiration that is printed on each vial.

**PROCEDURE:**

1. Aspirate vesicular fluid with needle and syringe and immediately place into UTM.
2. Unroof vesicle and collect fluid with a swab (non-Calcium alginate). Place in UTM.
3. Lesion scrapings: Scrape the base of an open vesicle with a sterile scalpel blade or rub base vigorously with swab. Place in UTM.

**TRANSPORT AND STORAGE:**

1. Take specimen to lab ASCP.

## **TZANCK PREPS FOR HERPES**

### **MATERIALS:**

1. Sterile scalpel
2. Frosted Slide
3. PAP fixative

### **PROCEDURE NOTE:**

1. This procedure is done in cytology and is included here for convenience. Call Cytologist or Pathology Secretary for results.

### **PROCEDURE:**

1. Scrape roof of lesion.
2. Smear on a small area of the slide.
3. Spray fix IMMEDIATELY with PAP fixative.
4. Label specimen. Fill out CYTOLOGY REQUEST FORM and deliver to lab IMMEDIATELY.

## **CHLAMYDIA-MYCOPLASMA OR UREAPLASMA UREALYTICUM CULTURES ARE SENT OUT TO A REFERENCE LABORATORY.**

### **MATERIALS:**

1. Chlamydia/N. gonorrhoeae:
  - A. BD ProbeTec ET Chlamydia trachomatis and Neisseria gonorrhoeae Amplified DNA Assay Collection Kit for Endocervical Specimens are available from the Send out department in the Laboratory.
2. Mycoplasma and Ureaplasma:
  - A. M 4 media available from the Send Out department in the Laboratory.

## **WET PREPS**

### **MATERIALS:**

1. Culturette

### **PROCEDURE NOTES:**

1. If specimen is also being submitted for culture, a separate specimen must be obtained using a culturette or appropriate transport as listed under GENITAL CULTURES.
2. Label specimen with patient's NAME, and DATE.
3. Wet preps are processed in Hematology.

### **PROCEDURE:**

1. Obtain specimen by collecting ample amounts of discharge from the vaginal wall.
2. Place swab in culturette and crush ampule

### **TRANSPORT AND STORAGE:**

1. Take specimen to lab IMMEDIATELY.
2. Keep at room temperature.

**AMNIOTIC FLUID, PERIVAGINAL OR DEEP PELVIC ABSCESES, TISSUE FROM MALE OR FEMALE INTERNAL GENITALIA**

**MATERIALS:**

1. Sterile syringe and needle for aspirates
2. BD Anaerobic Transport Vial
3. Sterile container for tissue specimens. These consist of tissue, fluid or purulent material.

**PROCEDURE:**

1. Collect specimen by aspiration if possible. See method under WOUNDS.
2. Place specimen in Anaerobic Transport following directions on the package.
3. Place tissue specimens in a sterile container.

**TRANSPORT AND STORAGE:**

1. Take specimen to Lab IMMEDIATELY.
2. Keep at room temperature.

## **GASTRIC FOR AFB**

### **MATERIALS:**

1. Levine tube
2. Syringe
3. Normal Saline
4. Sputum Collection System

### **PROCEDURE NOTES:**

1. Test is usually done for 3 consecutive days, unless the physician orders a single sample.
2. Gastric for AFB is done to concentrate AFB organisms in the stomach that are swallowed from the respiratory tract.

### **PROCEDURE:**

1. Patient preparation: NO smoking, food, or liquids after the evening meal.
2. Collect specimen in the morning
3. Pass a Levine tube into the stomach and withdraw all fasting contents. Place contents in the Sputum Collection System.
4. If no specimen is obtained, wash stomach by injecting and withdrawing 25-50 ml of Normal Saline 3-4 times. Withdraw and place in Sputum Collection System.

### **TRANSPORT AND STORAGE:**

1. Label specimen and deliver to Laboratory ASAP.
2. If there is any delay, refrigerate specimen.

## **INTRAVENOUS CATHETERS**

### **MATERIALS:**

1. Sterile hemostat from SPD
2. Sterile scissors from SPD
3. 2 Culturettes
4. Alcohol swabs or sponge
5. Sterile forceps
6. Sterile towel
7. Band-Aid
8. Extra culturette

### **PROCEDURE NOTES:**

1. One culturette is extra in case there is pus present after catheter removal.
2. Needed when removing long catheters.
3. Needed for tip of long catheters. Be careful not to brush the tip of removed catheter against the surrounding skin or environment.
4. Each specimen goes into separate culturette tube and should be properly identified.
5. The cotton at the bottom of the culturette should be moist and the tip should be resting on it.

**COLLECTION:**

1. Before removing the catheter, cleanse carefully around the insertion site with an alcohol swab to remove any residual antimicrobial ointment.
2. After the alcohol dries, carefully remove the catheter using the following technique:
  - A. Short plastic catheters: Holding the hub of the catheter in one hand, carefully withdraw catheter. Clamp the sterile hemostat at the tip of the catheter and sever the catheter aseptically 1/4-1/2 inch from the tip.
  - B. Butterfly needles: Carefully withdraw needle by holding on the plastic wings. Clamp the sterile hemostat at the tip of the steel needle and bend the hemostat back and forth until the needle snaps.
  - C. Long catheters (subclavian, Swan Ganz, CVP, etc.): Clamp catheter at junction where catheter enters skin and slowly remove catheter and put on sterile towel. With sterile scissors, cut 2 inches off the tip and 2 inches from the intracutaneous area just below the hemostat. (See diagram on next page)
3. Remove and discard the swab from one of the culturettes.
4. Aseptically place the catheter segment or needle into the culturette tube.
5. Crush the ampule at the bottom of the tube.
6. If pus can be expressed at the I.V. site after the removal of the needle or catheter, collect it and place in culturette as a separate culture.
7. Apply sterile Band-Aid over I.V. site.
8. Label specimens.

**TRANSPORT AND STORAGE:**

1. Take specimens to laboratory IMMEDIATELY.
2. Keep at room temperature.

## **PNEUMOCYSTIS STAINS**

**GENERAL INFORMATION:**

1. Diagnosis of *Pneumocystis carinii* is based on the actual demonstration of the organisms.
2. Open lung biopsy specimens are most likely to reveal the organisms.
3. Less invasive procedures such as broncho-alveolar lavage, bronchial brushes, and bronchial washes have a lower yield, but may be helpful when biopsy is contra-indicated.
4. Sputum specimens ARE NOT recommended.

**MATERIALS:**

1. Sterile container for specimen with NO PRESERVATIVE
2. Frosted clean Microscope Slides for Direct Smears

**PROCEDURE NOTE:**

Recovery is better from an "induced sputum specimen" than from an "expectorated sputum specimen."

**COLLECTION:**

1. Collect specimen (lung biopsy, bronch brushes, broncho-alveolar lavage, bronch wash).
2. Place specimen in sterile container.

3. For DIRECT SMEARS from biopsy specimens, or from cytology brush, the following instructions apply:
  - A. Place a small amount of the specimen on a clean frosted slide.
  - B. Spread the specimen thinly on an area about the size of a nickel.
  - C. Allow specimen to air dry.
  - D. Label slide.

**TRANSPORT AND STORAGE:**

1. Take specimen(s) and slides to Lab IMMEDIATELY.
2. Keep at room temperature.

## **RESPIRATORY CULTURES**

### **THROAT STREP SCREEN**

**MATERIALS:**

1. Culturette
2. Tongue Blade
3. Light source to illuminate throat

**PROCEDURE NOTES:**

1. Strep screens are used to distinguish Group A streptococcal infections from untreatable viral infections.
2. Label specimen

**PROCEDURE:**

1. Using a swab, with the patient's tongue depressed and the throat well-illuminated, vigorously rub the swab firmly over the back of the throat, both tonsils and tonsillar fossae and any other area of inflammation, exudation or ulceration.
2. Place swab in culturette, crush ampule.

**TRANSPORT AND STORAGE:**

1. Transport culturette to lab within 6 hours.
2. Keep at room temperature.

## **THROAT AND NASOPHARYNGEAL: DIPHTHERIA**

### **MATERIALS:**

1. 2 culturettes
2. Tongue Blade
3. Light Source to illuminate throat
4. Nasopharyngeal Swab (Rayon)

### **PROCEDURE NOTES:**

Cultures for DIPHTHERIA are sent to SouthCentral Regional Lab in Anchorage.

### **PROCEDURE:**

1. Using the technique described under Strep Screen, collect a throat swab.
2. Place swab in Culturette, crush ampule.
3. Using the procedure described under Nasopharyngeal, collect a NP swab.
4. Place NP swab in second Culturette and crush ampule.

### **TRANSPORT AND STORAGE:**

1. Take both Culturettes to the lab IMMEDIATELY. Keep at room temperature

## **THROAT: N.GONORRHOEA (GC)**

### **MATERIALS:**

1. Culturette
2. Tongue Blade
3. Thayer Martin/Chocolate Agar (ML/CA) pill pocket plate or Thayer Martin plate plus a Chocolate plate
4. CO2 pill and Ziplock bag
5. Light source to illuminate throat

### **PROCEDURE NOTES**

1. GC can die quickly. Proper use of CO2 pill pocket plates and immediate transport to lab facilitate optimum recovery.
2. Microbiology order form must include TIME OF COLLECTION, PATIENT'S AGE.

### **PROCEDURE:**

1. With tongue depressed and throat illuminated, swab the posterior pharynx and the region of the tonsillar crypts.
2. Roll the swab directly onto both media making sure that all parts of the swab that might have inoculum touch the media.
3. Label plate with patient's name.

### **TRANSPORT AND STORAGE**

1. Place CO2 pill into "pocket".
2. Place plate in Ziplock bag and seal tightly.
3. Take specimen to Lab IMMEDIATELY.
4. Alert Lab Assistant that there is a culture for GC so that specimen can be promptly taken to Microbiology.

## **NASOPHARYNX**

### **MATERIALS:**

1. Sterile Rayon Tipped Swab
2. Culturette

### **PROCEDURE NOTES:**

1. Results of NP cultures must be carefully interpreted. Presence of potential pathogens does not always indicate an infection.
2. Label specimen. Order in Cerner.

### **PROCEDURE:**

1. The specimen may be collected through the mouth by passing a NP swab under and beyond the uvula to the posterior wall of the nasopharynx to collect specimen through the nose, pass the wire swab along the floor of the nasal passage to the posterior wall.
2. Try to secure a bit of mucus by gently twirling swab while in place against the nasopharyngeal wall.
3. Place NP swab in Culturette and crush ampule.

### **TRANSPORT AND STORAGE**

1. Take specimen to Lab as soon as possible.
2. Keep at room temperature.

## **NASOPHARYNX FOR PERTUSSIS BY PCR AND CULTURE**

### **MATERIALS:**

1. Dacron Nasopharyngeal Swab in plain Dry Tube (Copan, yellow top)
2. Regan-Lowe (RL) transport media
3. Individually Paper-Wrapped nasopharyngeal Dacron/Polyester Swab
4. Pertussis Requisition Form

### **PROCEDURE NOTES:**

1. Pertussis Collection Kits are available in Microbiology Lab.
2. Testing for Pertussis is done at the State Lab in Anchorage. Specimens are sent via State Lab in Fairbanks.
3. Fill out State Microbiology Request Form.

### **PROCEDURE:**

1. Using the technique described under Nasopharyngeal, collect two swabs, one for PCR and the other for culture.
2. Place one swab back into its plain dry tube (yellow cap),
3. Place second swab into the Regan-lowel transport media by stabbing it into the media all the way to the bottom of the tube, break off swab.
4. Label specimens

### **TRANSPORT AND STORAGE:**

1. Keep specimens at room temperature.
2. Take specimens to lab IMMEDIATELY.

## **NOSE**

### **MATERIALS:**

1. Culturette

### **PROCEDURE NOTES:**

1. Potential pathogens in the nose can be part of the normal oral flora so that their presence does not always indicate an infection.
2. Nose cultures can be used to detect the carrier state of a pathogen.
3. If ordered as an aerobic culture, be sure to use the BD Anaerobic Transport Tube

### **PROCEDURE:**

1. Swab the anterior nares only.
2. Place swab in culturette and crush ampule. Label specimen.

### **TRANSPORT AND STORAGE:**

1. Take specimen to Lab ASAP.
2. Keep at room temperature.

## **EPIGLOTTIS**

### **MATERIALS:**

See Nasopharyngeal Culture

### **PROCEDURE NOTES:**

1. After an airway has been assured NP and Blood Cultures should be taken.
2. The epiglottis IS NOT recommended for culture in cases of acute epiglottitis because of the danger of causing an airway obstruction.

### **PROCEDURE:**

1. See procedure for NP culture.
2. Blood cultures are recommended.

## **MOUTH**

### **MATERIALS:**

1. Culturette
2. Tongue blade

### **PROCEDURE NOTES:**

1. Mouth cultures ARE NOT recommended EXCEPT for YEAST.
2. If abscess is present, see procedure for Superficial Wound.

### **PROCEDURE:**

1. Swab the affected areas of the mouth.
2. Place swab in Culturette, crush ampule.

## **SPUTUM**

### **MATERIALS**

1. Sputum Collection System (Materials Management) or Sterile Sputum Traps (Materials Management and Suction Machine (Respiratory Therapy))

### **PROCEDURE NOTES:**

1. First morning specimen is best because it takes advantage of secretions pooled overnight.
2. In order to maximize detection of ACID FAST BACILLUS, collect 3 early morning specimens on 3 different days.
3. COLLECTION PROCEDURE IS USED FOR ALL THREE OF THE FOLLOWING TESTS:
  - A. Routine culture (detects pneumococcal, staphylococcal, gram negative bacterial infections).
  - B. Fungal Culture (detects yeasts and filamentous fungi).
  - C. AFB Smear and Culture (detects Acid Fast Bacillus)
4. If the patient produces only saliva, repeat at a later time. If the patient consistently produces only saliva, notify the physician to see if she/he might order Respiratory Therapy to collect an induced specimen. Sputum specimens that have Routine Cultures ordered are evaluated microscopically for the presence of contaminating oral flora. [The presence of >25 epithelial/hpf indicates that the specimen is grossly contaminated with saliva. This results in a REJECTED specimen. Lab will contact nursing unit to request new specimen.](#)
5. AFB specimens are also evaluated. However, they will NOT be rejected by the Lab. The physician can however, take into account the quality of the specimen and order additional specimens BEFORE taking the patient off RESPIRATORY ISOLATION. [Submit minimum of 5 ml of sputum for AFB culture.](#)

### **PROCEDURE:**

1. Get sputum container out of package so that it is ready to use.
2. As soon as patient awakens in the morning, have him clear his throat and discard superficial phlegm.
3. Have patient breathe as deeply as he can several times.
4. Have patient cough as hard as he can from deep in the chest.
5. IMMEDIATELY have the patient place the specimen produced in the container without unduly holding it in his mouth.
6. Leave the entire sputum system intact. Tape lid closed.

### **TRANSPORT AND STORAGE:**

1. Take specimen IMMEDIATELY to Lab.
2. Keep refrigerated.
3. If the specimen has been collected in a "sputum trap," follow directions on the envelope. Label and take IMMEDIATELY to the Lab.

## **TRANSTRACHEAL ASPIRATES (TTA'S), BRONCHIAL BRUSHES, BRONCHIAL WASHINGS**

These specimens are collected by the Physician and nursing staff. Label specimen and IMMEDIATELY take to Lab. Bronchial Brushes can be used to isolate anaerobes. Therefore, prompt delivery (within 15 minutes) to the Lab is essential.

## **MASTOID SINUS CULTURE**

### **MATERIALS:**

BD Anaerobic transport vial

### **PROCEDURE NOTES:**

1. Since there is a possibility that the infection could be caused by anaerobes, use the BD anaerobic transport vial.
2. Anaerobic transport vials can be used for both aerobic and anaerobic cultures..

### **PROCEDURE:**

1. These specimens are usually collected by the physician in the OR.
2. Place specimen in the BD anaerobic transport vial following package directions.
3. Label specimen.

### **TRANSPORT AND STORAGE:**

1. Take specimen to the lab IMMEDIATELY.
2. Keep at room temperature.

## **PARANASAL SINUS CULTURE**

### **MATERIALS:**

1. Needle and syringe for aspirates
2. BD anaerobic transport vial

### **PROCEDURE NOTES:**

1. Anaerobic infections are possible in these sites.

### **PROCEDURE:**

1. The specimen is usually collected in OR by the physician. Care must be taken to use appropriate techniques to maintain viability of anaerobes.
2. Place specimen in BD anaerobic transport vial.
3. Label specimen.

### **TRANSPORT AND STORAGE:**

1. Take specimen to lab IMMEDIATELY.
2. Keep at room temperature.

## **RSV - RESPIRATORY SYNCYTIAL VIRUS ANTIGEN DETECTION**

### **MATERIALS:**

1. Saline
2. 3-5 ml syringe
3. 2" 18-20 gauge tubing
4. UTM - Universal Transport Media

### **PROCEDURE NOTES:**

1. RSV is the major cause of respiratory tract illness affecting young children.
2. Test can be ordered "stat". Turn-around time is 30 minutes.

### **PROCEDURE:**

1. Fill syringe with saline; attach tubing to syringe tip.
2. Quickly instill saline into nostril
3. Aspirate the recoverable nasal specimen. Recovery must occur immediately, as the instilled fluid will rapidly drain.
4. Inject aspirated specimen from syringe into UTM.
5. Vigorously mix specimen.

### **TRANSPORT AND STORAGE:**

1. Deliver to Lab IMMEDIATELY.

## **STOOL CULTURE**

### **ADULTS AND OLDER CHILDREN**

#### **RECOMMENDED GUIDELINES**

##### **MATERIALS:**

1. Clean container for collecting specimen such as a bedpan or wide cup. Saran wrap over the toilet can also be used for collection.
2. Enteric pathogen transport media (ETM; Cary-Blair formula) – AlphaTec ETM available from Materials Management or Microbiology Lab.

##### **PROCEDURE NOTES:**

1. Collection should be made without contaminating with urine.
2. Antibiotics and mineral oil are toxic to bacteria. DO NOT administer before collection.
3. 2-3 specimens collected on different days are sufficient for bacterial exam. To detect the carrier state, collect 3 specimens on separate days.
4. If diarrhea has commenced after the patient has been hospitalized 3 or more days, a routine stool culture or an Ova and Parasite examination is NOT recommended. Toxin assay for *C. difficile* may be indicated.
5. Routine Stool Culture include testing for the following pathogens:
  - A. Salmonella sp.
  - B. Shigella sp.
  - C. Campylobacter sp.
  - D. Shiga Toxins
  - E. Aeromonas sp.
6. Other pathogens or potential pathogens that can be detected by lab methods, but require special culture procedures or referral to reference laboratories are:
  - A. Yersinia enterocolitica
  - B. Vibrio sp.
  - C. Bacillus cereus
  - D. Listeria monocytogenes
  - E. Clostridia
  - F. Special requests must be made for these organisms. Call lab before collecting specimen.
7. Try to select portions that have blood or mucus.
8. Transport media will preserve specimen for several days. However, prompt delivery facilitates timely results.

##### **PROCEDURE:**

1. Have patient collect specimen in a suitable container.
2. IMMEDIATELY after patient has collected the specimen, transfer a portion to the Enteric pathogen transport media. Fill to line as directed on the vial.
3. Cap securely and mix vigorously. Label.

##### **TRANSPORT AND STORAGE:**

1. Take specimen to Lab ASAP.
2. Keep specimens in transport media at refrigerator temperature.

## **STOOL CULTURE: INFANTS IN DIAPERS**

### **MATERIALS:**

1. Diaper partially lined with nonpermeable liner such as plastic wrap.
2. Enteric pathogen transport media
3. Alternative method is to place a swab in the stool collected and then put the swab in CARY BLAIR TRANSPORT MEDIUM. Tubes should be kept in the dark at room temp. Make sure the swab is well submerged in the transport tube, then break off the top, and recap. Send to lab as soon as possible. CARY BLAIR TRANSPORT is available on the Pediatric Unit, or from the lab for other areas. Call 5647.

### **PROCEDURE NOTES:**

1. Diapers that are not lined tend to absorb most of a liquid or semiliquid stool. The powder, etc., may be toxic to the bacteria. Therefore, if it is possible, try to maximize collection with a non-absorbent, non-permeable liner placed strategically in the diaper.
2. Try to select portions that have mucus or blood.
3. Specimens in diapers are NOT ACCEPTABLE.
4. Transport media will preserve specimen for several days. However, prompt delivery facilitates timely results.

### **PROCEDURE:**

1. Place liner in diaper.
2. As soon as there is a specimen, transfer a portion to the Enteric pathogen transport media. Fill to line as directed on label.
3. If using CARY BLAIR TRANSPORT, recap tube after collection, and send to Lab at room temperature.

### **TRANSPORT AND STORAGE:**

1. Take specimen to Lab ASAP
2. Keep specimens in transport media at refrigerator temperature.

## **RECTAL SWABS FOR CULTURE**

### **MATERIALS:**

Culturette

### **PROCEDURE NOTES:**

1. Rectal swab specimens ARE NOT acceptable for Ova and Parasites (O&P), Gram Stain or Stool for WBCs.
2. Rectal swab specimens must reach lab within 1/2 hour. Specimens that are delayed are subject to changes in pH. If there is a change in pH, Shigella sp. can be lost.

### **PROCEDURE:**

1. Remove swab from Culturette tube. Pass swab beyond the anal sphincter, firmly rotate swab and withdraw.
2. Place swab in Culturette. Crush ampule. Label specimen.

### **TRANSPORT AND STORAGE:**

1. Take Culturette to Lab IMMEDIATELY.
2. Keep at room temperature.

## **STOOL FOR GRAM STAIN OR WBCs (POLYS)**

### **MATERIALS:**

1. Clean container for collecting specimen such as bedpan or wide cup. Saran wrap over the toilet can be used for collection.
2. Specimen cup with tight lid for transport

### **NOTES:**

1. This IS NOT an acceptable specimen for Culture or for Ova and Parasites
2. If possible, try to transfer any portion that contains mucus or blood.
3. For GRAM STAIN, fill out Microbiology Request form.
4. For WBCs, fill out ROUTINE Lab Request Form.
5. Stool for WBCs is done in Hematology.

### **COLLECTION:**

1. Have patient collect specimen.
2. Transfer a portion into specimen cup. Label.

### **TRANSPORT AND STORAGE:**

1. Take specimen to Lab IMMEDIATELY.
2. Place specimen in refrigerator upon arrival in lab.

## **STOOL FOR CLOSTRIDIUM DIFFICILE**

### **MATERIALS:**

See "Stool for Gram Stain"

### **PROCEDURE NOTES:**

1. Toxin assays are preferred for detection of C. difficile. Culture is rarely requested. If both are requested, send at least a 15cc portion of stool. C. Difficile toxins are done here at FMH. If culture is requested, it is sent to Quest reference lab.

### **COLLECTION:**

1. Have patient collect specimen.
2. Transfer a small portion to a gray container or a clean specimen cup. Label.

## **STOOL FOR OVA AND PARASITES**

### **MATERIALS:**

1. Clean container for collecting specimen
2. O&P Transport Media -1 vial of Zn-PVA Fixative and 1 vial of 10% Buffered Neutral Formalin (Together as Para-Pak)

### **PROCEDURE NOTES:**

1. Patient can collect and transfer own specimen if able to do so. Instruct patient in collection and transfer procedure by using pamphlet insert that comes with the transport vial.
2. Try to select portions that are bloody or have mucus.
3. To maximize detection of parasites, ORDER O&P X 3. Since some organisms are shed in a variable pattern, it is recommended that specimens be collected every **SECOND** or **THIRD DAY**. In the hospital, one specimen each day for three days is acceptable.
4. Transport media will preserve O & P specimen for weeks. However prompt delivery facilitates timely results.
5. O&P is now a send-out to Quest.

### **COLLECTION:**

1. Have patient collect specimen in bedpan or clean cup.
2. Transfer portion of stool to the vial. Fill to line as directed on label of vial.
3. Mash or stir specimen in liquid until is well mixed with fluid.
4. Replace cap **TIGHTLY**. Shake **HARD** until mixture looks like soup.
5. Label specimen.

### **TRANSPORT AND STORAGE:**

1. Take to Lab as soon as possible.
2. Keep at room temperature.

## **STOOL FOR GIARDIA ANTIGEN ONLY**

### **MATERIALS:**

Stool specimen can be collected in the O & P vial, or Enteric transport as for Stool culture or in a gray container with no preservative, if kept refrigerated.

### **PROCEDURE NOTE:**

Transport to Lab as soon as possible. Specimen will be processed the same day if received in the lab by 12:30 PM.

## **PINWORM PREP**

### **MATERIALS:**

Sterile Pinworm Paddle (available in Microbiology Lab)

### **PROCEDURE NOTES:**

Handle specimens very carefully. Pinworm eggs are easily transmissible.

### **PROCEDURE:**

1. Obtain the specimen early in the morning before the patient has arisen.
2. Remove cap in which is inserted a clear polystyrene paddle with one side coated with a non-toxic mildly adhesive material. This side is marked "sticky side". Do not touch this surface with fingers.
3. Press the sticky surface against the perianal skin with moderate pressure.
4. Place cap back into container. Label specimen.

## **URINE**

### **GENERAL INFORMATION:**

1. First morning midstream clean-catch urine is best for culture. Otherwise obtain a specimen in a minimum of 2 hours after last voiding.
2. Since urine is an excellent medium for growth of bacteria, and the colony count is used for deciding whether the patient has UTI, the urine must be taken to the Lab IMMEDIATELY after collection. Refrigerate (2-8 C) the specimen if there is a delay in transport.
3. Specimens that have been delayed in being placed in the BD gray-top transport tube for more than 1/2 hour and have not been refrigerated are NOT ACCEPTABLE for culture.

### **URINE—ROUTINE CULTURE INFANTS**

### **PROCEDURE NOTES:**

1. Refrigerated specimens can be cultured up to 24 hours after collection.
2. Be sure to label specimen. Place in a plastic urine cup for easier handling.
3. Specimen must be accompanied by a properly filled out Microbiology Request Form.

### **MATERIALS:**

1. Pediatric Urine Collection Kit (from Materials Management)
2. Wash cloth
3. Soap and Water

### **PROCEDURE:**

1. Wash genital area using wash cloth with soap and water.
2. Apply Pediatric urine collection bag.
3. Check frequently to see if specimen has been collected.

### **TRANSPORT AND STORAGE:**

1. When specimen is obtained, take to Lab IMMEDIATELY.
2. If there is any delay in transport, refrigerate specimen.
3. Place specimen in refrigerator when delivering to lab.

## **URINE ADULT COLLECTION**

### **MATERIALS:**

1. Clean Catch Collection Kit (from Materials Management) containing:
  - A. 1 screw-cap specimen cup with sampling device
  - B. 1 red top Vacutainer conical tube for urinalysis
  - C. 1 gray top Vacutainer tube for culture
  - D. 2 cleansing Towelettes
2. Chair or shelf in bathroom to place collection kit on.

### **PROCEDURE NOTES:**

1. Be careful NOT to touch the inside of the collection cup or the inside of the lid.
2. After use, Towelettes are to be discarded into the toilet.

### **PROCEDURE—FEMALE:**

1. Have patient remove undergarments, wash hands with soap and water.
2. Open collection kit. Unscrew cap of the urine specimen cup. Place cap on shelf with "straw" facing upward. DO NOT TOUCH INSIDE OF CUP, CAP OR STRAW. Have Towelettes within reach along with collection cup.
3. With one hand, the patient is to separate her labia and wipe the inner folds front to back in a single motion
4. Using the Towelettes, the patient is to wipe along one side of the opening (meatus) from front to pack. Take second towelette and repeat down other side. Discard. Take third towelette and wipe down the middle. Discard.
5. Pass a small amount of urine into toilet (or bedpan), and without stopping stream, hold specimen cup a few inches from opening and catch remaining urine. DO NOT overflow cup.
6. Place lid securely on specimen cup so that it does not leak.

### **PROCEDURE—MALE:**

1. Remove lid from specimen sup.
2. Have towelette ready.
3. Pull back the foreskin to expose glans (if indicated). Wipe away from the opening (urinary meatus). Discard towelette in toilet.
4. Allow initial urinary flow to drain into urinal or toilet and without stopping stream, hold specimen cup a few inches from penis and catch remaining urine. DO NOT overflow cup.
5. Place lid securely on specimen so that it does not leak.

### **TRANSPORT AND STORAGE:**

1. Take specimen to lab IMMEDIATELY.
2. Keep at refrigerator temperature.
3. Place specimen in refrigerator upon delivery to lab.

## **URINE—FOLEY CATHETERS**

### **COLLECTION FOR INDWELLING FOLEY CATHETERS**

#### **MATERIALS:**

1. Foley clamp
2. Sterile syringe and needle
3. Sterile tube
4. Alcohol sponge

#### **PROCEDURE NOTES:**

1. If specimen is also needed for Urinalysis, a 10-20ml syringe is needed.
2. If Urinalysis is also requested, withdraw 10-20ml.

#### **COLLECTION:**

1. Clamp off drainage tube below bifurcation of tubing for 15-20 minutes. DO NOT DISCONNECT.
2. Cleanse Foley with alcohol. Withdraw a minimum of 2ml of urine for culture. Be sure to insert needle into Foley BELOW bifurcation, unless tubing has an area especially for this purpose, in which case, clamp off the drainage tube instead of the Foley.
3. Place urine in sterile tube.
4. Label specimen.

#### **TRANSPORT AND STORAGE:**

1. Take specimen to lab IMMEDIATELY.
2. Keep at refrigerator temperature.
3. Place specimen in refrigerator upon delivery to lab.

## **URINE—AFB**

#### **MATERIALS:**

Clean container for collecting urine

#### **PROCEDURE NOTES:**

24 hour specimen is NOT acceptable.

A direct smear for AFB is not done on urine specimens.

Early morning specimen is preferred to take advantage of pooling of organisms.

#### **PROCEDURE:**

1. See procedure under URINE CULTURE for Clean Catch Procedure.
2. Send a minimum of 15 ml in a sterile cup.

#### **TRANSPORT AND STORAGE:**

1. Transport to Lab ASAP.
2. Refrigerate specimen if there is any delay.

## **URINE—ANTIGEN TESTING**

Urine antigen testing is no longer performed at FMH. If requested, test will be send to a reference laboratory.

## **URINE—SUPRAPUBIC ASPIRATES (SPA)**

### **MATERIALS:**

1. Iodine and alcohol sponges
2. 10 ml syringe, 22 gauge needle
3. Sterile tube and/or BD Anaerobic Vial

### **PROCEDURE NOTES:**

1. Suprapubic aspirates are done so that collection can be free of contaminating genital flora. It is also the only urine specimen that is acceptable for anaerobes.
2. Infants may require a smaller syringe and needle.
3. Deliver to Lab immediately and process ASAP.

### **PROCEDURE:**

1. Procedure is done by physician.
2. Set up routine urine culture plus an anaerobic plate.
3. All suprapubic aspirates are set up for aerobes and anaerobes.

### **TRANSPORT AND STORAGE:**

1. Take specimen to lab IMMEDIATELY.

## **VIRAL CULTURES**

### **GENERAL INFORMATION:**

1. Viral cultures are sent to the State Virology Lab located on the UAF campus (phone 474-7017).
2. Collection instructions are taken from the State Virology Laboratory Service Manual, published by the Alaska Department of Health and Social Services, 2004.
3. Generally, viral specimens **MUST BE** collected within the acute phase of infection.
4. A longer interval between illness and onset is acceptable for the following:
  - A. Rectal swab/feces for enteroviral culture
  - B. Urine culture for CMV, mumps, culture.
  - C. Vesicular fluid, CSF, biopsy specimens.
5. State Virology Laboratory must have **COMPLETED** Virology Request Form before they process specimens.
6. Label specimens with patient's NAME, DATE, IDENTIFICATION NUMBER and source.

### **MATERIALS:**

1. Universal Transport Media (UTM) available at the laboratory send out desk.
2. Sterile Synthetic Swab (for throat, rectal, vesicle, lesion, or eye collection)
3. Sterile Rayon Tipped Swab (for NP, eye collection). **DO NOT USE WOODEN SHAFT OR COTTON SWABS.**
4. Tuberculin syringe with 26 or 27 gauge needle (for vesicle fluid collection)
5. Isotonic salt solution PBS (for pharyngeal or NP washing)
6. Sterile containers (for collection of urine, feces, or pharyngeal washing)
7. Rubber bulb syringe (for NP washing)

### **PROCEDURE NOTES:**

1. RSV's (Respiratory syncytial virus) are done at FMH only if the Physician wants a STAT result. RSV's can be done at the State Lab as part of a Viral Culture and they are run once a week. Rotavirus tests are done at the State Lab.
2. A copy of this manual is available in the Microbiology Laboratory.
3. Respiratory viruses, herpesvirus and Varicella Zoster virus decrease in recovery rate after 5-6 days.
4. Virology Request Forms are available from the Microbiology Lab.
5. UTM is good until the expiration date on the vial.

### **COLLECTION, STORAGE, AND TRANSPORT:**

#### **1. PHARYNGEAL WASHING:**

- A. Introduce saline (PBS) into patient's mouth with a drinking glass or syringe (without a needle). With patient's head retracted, have patient gargle fluid. Collect fluid in a sterile container. Repeat procedure for at least 3 minutes. Place fluid in UTM. Take labeled specimen to Lab with Viral Request Form.
- B. Total volume of PBS should not exceed 20 ml.

#### **2. NASAL WASHING:**

- A. Tilt the patient's head back an angle of about 70. Insert rubber bulb syringe (1 oz. tapered containing 3-7ml of PBS) until it occludes the nostril. Collect specimen with one complete squeeze and release bulb. Place contents in UTM. Take labeled specimen to Lab with Viral Request Form. .
- B. Nasopharyngeal washing are superior to swabs.

- C. Nasopharyngeal washings are superior for respiratory syncytial virus (RSV) influenza, and parainfluenza viruses.
- D. Nasal washings are submitted for RSV antigen tests done at FMH. Fill out routine Lab Slip and submit with specimen. See procedure under RSV.
- 3. **THROAT SWABS:**
  - A. Material is obtained by rubbing the oropharynx vigorously with a synthetic (rayon) swab. Include material from the posterior pharynx, tonsils, faucial pillars and/or any inflamed erythematous areas or visible lesions. Place swab in UTM. Take labeled specimen to Lab with Viral Request Form.
  - B. Throat swabs are adequate for recovery of entero and adenovirus isolation.
- 4. **NASAL SWABS:**
  - A. Collect the specimen by inserting a flexible wire swab into the nasopharynx just posteriorly to the turbinate. Rotate the swab, remove, and place in UTM. Take labeled specimen to Lab with Viral Request Form.
  - B. Nasal specimens are optimal for rhinovirus recovery.
- 5. **FECAL SPECIMENS:**
  - A. Place 1-2 (<1 tsp) sample into UTM. Take labeled specimen to Lab with Viral Request Form.
  - B. Most cases of viral gastroenteritis are due to viruses that cannot be grown in culture, e.g., rotavirus, Norwalk agents, and some adenoviruses, but can be demonstrated by enzyme immunoassay (EIA).
- 6. **RECTAL SWAB:**
  - A. Use UTM to moisten 2 sterile synthetic (rayon) swabs and insert well into the rectum (about 3-5cm). The mucosa is rubbed until fecal material adheres onto the swab. Place swab in UTM. Take labeled specimen to Lab with Viral Request Form.
  - B. Generally, stool specimens are more productive than rectal swabs.
- 7. **CSF, PERICARDIAL OR PLEURAL FLUID:**
  - A. 3 ml of fluid should be collected under aseptic conditions. Store in sterile tubes. Take labeled specimen to Lab with Viral Request Form. Keep refrigerated.
  - B. If possible, collect specimens Sunday through Thursday. Because there is a delay in delivery to the State Lab for those specimens that are collected on Friday and Saturday, there may be some loss of viability of the virus.
- 8. **VESICLE FLUID:**
  - A. The area around the lesion is cleaned with ether or acetone. Aspirate the fluid into a tuberculin syringe (about 0.2ml). Place aspirate into UTM. Rinse syringe with UTM and discharge fluid back into UTM vial.
  - B. Alternatively, rupture vesicle and absorb fluid onto synthetic (rayon) swabs by gently rubbing the vesicle base with the swab. Place swab in UTM.
  - C. Take labeled specimen to Lab with Viral Request Form.
  - D. Fluid and cells from vesicles are superior to specimens recovered from ulcers for culture and for staining.
- 9. **DERMAL/GENITAL LESIONS:**
  - A. The vesicle may be ruptured and/or the surface of the lesions scraped to obtain vesicle fluid and cells from the base of the lesion using a swab. Place specimen in UTM. Take labeled specimen to lab with a Viral Request Form.
- 10. **URINE:**
  - A. First morning clean voided urine preferred. Collect at least 10 ml in a sterile container. Take labeled specimen to Lab with Viral Request Form. Keep refrigerated.

- B. Mumps, adenovirus, and CMV can be recovered from urine. Two or three specimens on successive days are suggested for maximum recovery of virus.

**11. EYE:**

- A. Conjunctiva:
  - 1. Press a swab moistened with UTM firmly against the inflamed area of the conjunctiva. Place swab in UTM.
- B. Corneal lesions:
  - 1. Corneal scrapings are collected by an ophthalmologist. Place material in UTM.
- C. Take labeled specimen to lab with Viral Request Form.
- D. An NP swab (Culturette or rayon) can be used to obtain secretions from the conjunctiva.
- E. Eye swabbing should be obtained by an ophthalmologist.

**12. TISSUE:**

- A. Take the biopsy sample from areas directly adjacent to affected tissue. Suspend tissue in UTM. Take specimen to Lab with Viral Report Form.
- B. Tissue and cells grown in cell culture subsequent to dispersal of the cells have resulted in higher virus recovery; e.g. lung (CMV, influenza, and adenoviruses), and brain (HSV). The cytological detection of CMV inclusions is 3-6x less sensitive than viral isolation; e.g., bronchial washing may contain inclusion-bearing cells in only 50% of specimens from which CMV is recovered.

**13. WHOLE BLOOD:**

- A. Draw a venous sample (heparinized for CMV, and clot for arbovirus) during the early acute phase of infection. Take labeled specimen to Lab with Viral Report Form. Keep refrigerated.
- B. Buffy coat cells from heparinized blood are occasionally useful for detection of CMV viremia in immunocompromised patients.

## **FLU A & B—INFLUENZAE A & B VIRAL ANTIGEN TEST**

### **MATERIALS:**

1. Saline
2. 3-5 ml syringe
3. 2" 18-20 gauge tubing
4. UTM (Viral Transport Media)

### **PROCEDURE NOTES:**

1. Flu A + B is a rapid EIA test for the detection of influenzae A and B viral antigen.
2. Test can be order "stat". Turn-around time is 30 minutes.

### **PROCEDURE:**

1. Fill syringe with saline: attach tubing to syringe tip.
2. Quickly instill saline into nostril.
3. Aspirate the recoverable nasal specimen. Recovery must occur immediately, as the instilled fluid will rapidly drain.
4. Inject aspirated specimen from syringe into UTM.
5. Vigorously mix specimen.

### **TRANSPORT AND STORAGE:**

1. Deliver to lab IMMEDIATELY

## **WOUNDS**

### **MATERIALS**

1. Culturette (for AEROBIC CULTURE ONLY)
2. BD Anaerobic Vial (for AEROBIC and ANAEROBIC)
3. Sterile syringe, needle
4. Sterile container (for curettage or biopsy)
5. Antiseptic swabs

### **PROCEDURE NOTES:**

1. Superficial wound infections are caused by AEROBIC bacteria. Culturette transport is suitable for transport.
2. Chronic open wounds such as sinus tracts, fistulae, incision, dehiscence, etc., are cultured AEROBICALLY and ANAEROBICALLY if specimen collection and transport is appropriate. Use BD Anaerobic Transport Tube.
3. Specimens received in CULTURETTES will be processed for AEROBES only.
4. Deep wounds should be cultured both AEROBICALLY and ANAEROBICALLY. Please take care to collect and transport as specified to preserve anaerobes.
5. Specimens received in Culturettes are NOT ACCEPTABLE for anaerobic culture.
6. Open deep wounds include post operative wounds, and recently opened abscess cavities.
7. Swab specimens are not recommended because they decrease anaerobe recovery.

### **COLLECTION-SUPERFICIAL WOUNDS**

1. Cleanse skin with antiseptic.
2. Collect specimen on culturette swab, sampling the deepest portion or active margin of the wound. Avoid contact with skin. DO NOT sample areas of healing.
3. Place swab in culturette, crush ampule.

### **COLLECTION FOR CHRONIC OPENED WOUNDS**

1. Cleanse surface with antiseptic.
2. Preferred specimen: Collect by curettage or biopsy of the wall of the wound.
3. Place specimen in sterile container.

### **COLLECTION FOR DEEP WOUNDS:** CLOSED deep wounds and abscesses.

1. Clean surface with antiseptic.
2. Aspirate with needle and syringe, being careful to sample deepest portion or active margin of the wound.
3. If little fluid is present, inject sterile saline into the site and re-aspirate.
4. Place aspirate into BD anaerobic vial following directions on the package.

### **OPEN DEEP WOUNDS:**

1. If there is enough liquid purulent material and the technique can be performed with minimal patient risk, aspirate the deepest portion or active margin of the site.
2. Place aspirate in BD Anaerobic Vial .

### **TRANSPORT AND STORAGE:**

1. Label specimen, take to Lab IMMEDIATELY.
2. Keep at room temperature.

**MRSA SURVEILLANCE CULTURES** (Methicillin Resistant Staphylococcus aureus)  
**VRE SURVEILLANCE CULTURES** (Vancomycin Resistant Enterococcus)  
**ESBL SURVEILLANCE CULTURES** (Extended Spectrum Beta Lactamase Organism)

**MATERIALS:**

1. Culturette

**PROCEDURES NOTES:**

Implementation of surveillance cultures to identify colonized patients and use of contact precautions for care of colonized patients prevents contamination of health care workers, apparel, and equipment and have been followed by a significant reduction in the rates of both colonization and infection of the patients with MRSA, VRE and ESBL.

**PROCEDURE:**

- **MRSA:** Collect a nasal, groin, and original site (unless it was a CSF, Blood or it was a wound that already healed). Collect 72 h after completion of antibiotics and for 2 consecutive days.
- **ESBL:** Collect a culture from the original source (unless it was a CSF, Blood or it was a wound that already healed) Collect 72 hours after completion of antibiotics for 2 consecutive days.
- **VRE:** Collect fecal specimen or rectal swab, and original site (unless it was a CSF, Blood or it was a wound that already healed). Feces show greater yield than perirectal swab. Swabs of wounds or urine may also be submitted. Collect 72 h after completion of antibiotics, and for 2 consecutive days.

**TRANSPORT AND STORAGE:**

1. Take to lab immediately.
2. Keep at room temperature.

## **MRSA SCREEN**

### **MATERIALS:**

1. Culturette

### **PROCEDURE NOTES:**

1. Patients admitted to ICU, 2S, and Denali Center will be tested within 1 hour of admission
2. All elective pre-op patients scheduled for surgery will be tested at more than 48 hrs and less than 30 days **prior** to surgery
3. All patients transferred to 2S and ICU will be tested
4. All patients in ICU with negative MRSA will be tested weekly
5. Results will be reported within 18-24 hours.

### **COLLECTION:**

Using a CultureSwab collect a specimen from the nares, groin or axilla.

### **TRANSPORT AND STORAGE:**

Take specimen to lab immediately

Store at room temperature

### **Annual Review**

<b>Microbiology Supervisor</b>	
<b>Signature</b>	<b>Date</b>